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Telomere DNA in the insect order Dermaptera and the first evidence for the non-canonical telomeric motif TTCGG in Arthropoda

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Abstract

Despite recent advances in telomere research, the telomere DNA organization remains unknown for representatives of several insect orders. In this study, analysis of the chromosome-level genome assembly shows that the telomeric DNA of the earwig *Labia minor* (Linnaeus, 1758) (Polyneoptera, Dermaptera, Spongiphoridae) consists of repeats of the 5 bp motif TTCGG/CCGAA. This is the first record describing the structure of telomeric DNA in the order Dermaptera. This record expands the spectrum of the known telomeric sequences, since the TTCGG motif has not been reported for insects previously.

Keywords

Chromosomes, Dermaptera, genome assemblies, insects, telomere, telomeric motif, Zoraptera

Introduction

In many insects, the organization of telomeric DNA is highly conservative and, with a few exceptions, telomeric DNA is represented by a short TTAGG motif, which is repeated tens, hundreds, and even thousands of times at the ends of chromosomes. This telomeric motif is considered to be ancestral for the class Insecta and the entire phylum Arthropoda and is preserved in the majority of the studied insect orders and families (Frydrychová et al. 2004; Vítková et al. 2005; Lukhtanov and Kuznetsova 2010; Kuznetsova et al. 2020).

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Recent studies have confirmed the widespread and frequent occurrence of the TTAGG motif (Gokhman and Kuznetsova 2018; Grozeva et al. 2019; Prušáková et al. 2021; Lukhtanov and Pazhenkova 2023; Bugrov et al. 2024). At the same time, they have shown that insects are characterized by a much greater diversity in the organization of telomeric DNA than was previously assumed. It has been shown that in addition to the canonical TTAGG motif, insects have a large number of other variants of telomeric repeats, the length of which varies from 1 to 11 nucleotides (Lukhtanov 2022; Zhou et al. 2022; Fajkus et al. 2023; Lukhtanov and Pazhenkova 2023; Lyčka et al. 2024; Stoianova et al. 2024). In some representatives of the Diptera families Syrphidae and Tachinidae, longer (173–381 bp) repeats are found at chromosome ends (Lukhtanov and Pazhenkova 2023). It has been also established that in most insects the arrays of the short telomeric motifs are interspersed with telomere-specific non-LTR retrotransposons of the SART and TRAS families (Kubo et al. 2001; Fujiwara et al. 2005; Osanai et al. 2006; Kirkness et al. 2010; Monti et al. 2013; Lukhtanov and Pazhenkova 2023; Pazhenkova and Lukhtanov 2023).

Despite the above-mentioned advances in the study of telomeric DNA in insects, the structure of telomeres remains unknown for representatives of many orders. This complicates the reconstruction of the ancestral organization of insect chromosomes and the study of the evolution of mechanisms that maintain telomere length. In particular, there are still no data on the organization of telomeric DNA in earwigs (the order Dermaptera) (Kuznetsova et al. 2020). This order is especially intriguing from the point of view of telomere organization. An attempt has been made to elucidate the structure of telomeric motif in the earwig *Forficula auricularia* Linnaeus, 1758 using the fluorescence *in situ* hybridization (FISH) method and the TTAGG probe (Frydrychová et al. 2004); however, it was unsuccessful. Two attempts to elucidate the telomere structure in earwigs using Southern blot hybridization (Okazaki et al. 1993; Frydrychová et al. 2004) were also unsuccessful. These attempts can be interpreted as evidence that the TTAGG motif is absent from the telomeres of the studied species. At the same time, they did not bring us any closer to understanding how telomeres are organized in earwigs.

In this study an analysis of the chromosome-level genome assembly of *Labia minor* (Linnaeus, 1758) (Spongiphoridae) and scaffold-level genome assemblies of *Forficula auricularia* (Forficulidae), *Euborellia annulipes* (H. Lucas, 1847) (Anisolabididae), and *Anisolabis maritima* (Bonelli, 1832) (Anisolabididae) was carried out to enable investigation of the telomeric DNA of Dermaptera.

Material and methods

Chromosome-level genome assembly of *Labia minor* generated by the Darwin Tree of Life Project (https://www.darwintreeoflife.org/) (2022) and freely available upon deposition in the European Nucleotide Archive (ENA) (https://www.darwintreeoflife.org/wp-content/uploads/2020/03/DToL-Open-Data-Release-Policy-1.pdf) was used for search and analysis of telomere and subtelomere sequences. Nucleotide sequences representing each of the seven chromosomes of this species were downloaded from GenBank (Table 1). Their terminal regions were visually inspected.

Table 1. The structure of the terminal ends in chromosomes of *L. minor*.

Chromosome	Left telomere	Right telomere	Chromosome size (bp)	GenBank
1	truncated	truncated	126 263 735	OY720344.1
2	truncated	truncated	95 403 478	OY720345.1
3	**CCGAA**	**TTCGG**	86 446 126	OY720346.1
4	truncated	truncated	83 479 239	OY720347.1
5	truncated	**TTCGG**	70 759 738	OY720349.1
6	truncated	truncated	60 004 694	OY720350.1
X	truncated	**TTCGG**	76 888 567	OY720348.1

In addition, scaffold-level genome assemblies *Forficula auricularia*, *Euborellia annulipes*, and *Anisolabis maritima* were examined. Telomere-like sequences in these genomes were searched using the GenBank *blastn* function with the "Low complexity region" filter disabled and the $(TTAGG)_{30}$ and $(TTCGG)_{30}$ sequences as queries.

Results and discussion

In the analyzed assembly, I found no evidence of telomeric motifs in chromosomes 1, 2, 4, 6 and in the left ends of chromosomes 5 and X (Table 1). This situation is not unexpected. Telomeric sequences at chromosome ends are the most difficult genome regions to assemble with accuracy and completeness (Rhie et al. 2021; Kim et al. 2022). Therefore, some assemblies may be truncated at the ends of chromosomes or even contain no telomeric repeats at all. Even if telomeric repeats are present, it is difficult to be sure that these sequences are complete and contain the correct number of copies.

However, if telomeric motifs are conserved on some chromosomes or at the ends of some chromosomes, they can be easily recognized by a combination of the following features. (1) Each telomeric end (if conserved in whole or in part) is represented by a large number of exactly identical short repeats. (2) These end structures, if present in the assembly, consist of the same motifs on different chromosomes. (3) If telomeric motifs are present at the left and right ends of the chromosome, they have a reverse-complement structure and orientation. (4) Short telomere-like motifs are rare in interstitial positions of chromosomes, and if they occur there, then in a small number of copies.

In the case of the *L. minor* chromosome assembly, the TTCGG telomeric motif was found in the most terminal positions of the right ends of chromosomes 3, 5 and X. In addition, the CCGAA motif, which is a reverse-complement sequence to TTCGG, was found in the terminal left end of chromosome 3 (Table 1). Each of these terminal sequences was represented by a large number of TTCGG/CCGAA repeats (from 351 in chromosome 5 to 481 in the left end of chromosome 3). In interstitial positions of all chromosomes, even short $(TTCGG/CCGAA)_3$ and $(TTCGG/CCGAA)_4$ repeats were rare. Based on this, I interpret the long sequences of short TTCGG/CCGAA motifs at the ends of chromosomes 3, 5, and X as telomeric sequences. The probability that these sequences represent a random combination of non-telomeric nucleotides seems vanishingly small.

In addition, I studied the subtelomeric regions of chromosomes bordering telomeric sequences. This analysis showed that the subtelomeric regions are rich in variant repeats of TTCAGTT and TTCGGTT. The presence of short repeats that are close in structure to telomeric motifs, but are slightly altered, was previously noted as one of the characteristic features of subtelomeric regions (Louis and Vershinin 2005). For the species *Forficula auricularia*, *Euborellia annulipes*, and *Anisolabis maritima*, in the GenBank, there are only scaffold-level genome assemblies that include no assembled chromosomes. A blast of these assemblies using the $(TTCGG)_{30}$ sequence as a query revealed that these assemblies contained extended arrays of $(TTCGG)_n$. Blast of these assemblies using the $(TTAGG)_{30}$ sequence did not reveal extended arrays of $(TTAGG)_n$. This result suggests that the TTCGG telomeric motif is most likely present in these species and is therefore widespread in the order Dermaptera.

The obtained result seems interesting in several respects. It is the first result showing the structure of telomeric DNA in the order Dermaptera. In addition, it expands the spectrum of the known telomeric sequences, since the TTCGG motif has not been reported for insects previously. Thirdly, this motif may be a synapomorphy marking either all the studied families (Spongiphoridae, Forficulidae and Anisolabididae), or all earwigs, or even a phylogenetic lineage of a higher rank. Earwigs belong to a monophyletic group of orders called Polyneoptera. For other orders of this group, the presence of the canonical telomeric motif TTAGG has been shown (Kuznetsova et al. 2020). The presence of the TTCGG motif may be a feature indicating an isolated position of earwigs. The order Dermaptera is sister to the enigmatic and poorly studied order Zorapera (Wipfler et al. 2019). Therefore, it would be interesting to elucidate the telomere organization in Zoraptera in the future.

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